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13. ABSTRACT (Maximum 200 Words) Corneal epithelial damage thresholds for exposures to sequences of pulses of $1.55 \mu\text{m}$ infrared radiation produced by an Er fiber laser were investigated. Thresholds were determined for sequences of 8 to 128 pulses at a repetition frequency of 10 Hz and 8 to 256 pulses at 20 Hz. The duration of the individual pulses was 0.025 sec and the 1/e diameter of the laser beam was 0.1 cm. Threshold damage is correlated by an empirical power law of the form $H_{th} = CN^{-\alpha}$, in which H_{th} is the threshold radiant exposure per pulse, and N is the number of pulses. The value of the exponent α is 0.32 for the 10 Hz exposures and 0.34 for the 20 Hz exposures. The constant C is 7.60 for the 10 Hz exposures and 5.99 for the 20 Hz exposures. Both values are greater than the estimated threshold for a single 0.025 sec pulse (4.90 J/cm^2). Thus the empirical power law breaks down for small numbers of pulses (viz., $N < 8$) where it overestimates the damage thresholds. An empirical critical temperature model also correlates the multiple-pulse injury thresholds.			
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--Introduction--

The research performed under this contract directly supports the U.S. Army Medical Research and Materiel Command (USAMRMC) mission to assess the health effects and hazards of non-ionizing electromagnetic radiation from laser systems. The data obtained will support evaluation of current permissible exposure limits promulgated by TEMED 524 and the ANSI Z-136 laser safety standards. The research addresses three main hypotheses: 1) Damage from 1.55 μm radiation is thermal; 2) Damage from sequences of pulses is cumulative and is correlated by a power law relating the threshold irradiance to the number of pulses in the sequence; and 3) exposures only slightly above the damage threshold for the corneal epithelium will result in damage to the corneal endothelium for these penetrating wavelengths. The hypotheses will be tested by: 1) determining corneal epithelial damage thresholds for single- and multiple-pulse exposures as functions of irradiance, exposure duration, and beam size for wavelengths near 1.55 μm . 2) developing and validating damage models for these wavelengths, and 3) determining thresholds for endothelial damage for single-pulse exposures as functions of irradiance, exposure duration, beam size, and position on the cornea and investigating the healing response for exposures above the epithelial damage threshold.

--Body--

Methodology

M1 - Laser System

An IPG Photonics ELD-10-1550 fiber laser purchased during Year 1 is now used for the exposures. This laser emits mid-infrared radiation at a wavelength of 1.55 μm . Its output is collimated and has an accurate Gaussian profile (1/e beam diameter 0.33 cm). The beam was focused with a 101 mm focal length biconvex glass lens. Corneas were positioned past the focus in order to vary the diameter of the exposed area. Mode quality was verified by direct viewing of the beam on a fluorescent screen. The beam was profiled with a knife-edge at the position where the cornea would be located at each experimental session to verify the Gaussian profile and to determine the 1/e beam diameter.^{1, 2} The laser is equipped for external pulse modulation to facilitate the multiple-pulse experiments. LabView software was written to control pulse duration, number of pulses, and pulse frequency. We built a reversed biased circuit for a (borrowed) Fermionics Model FD2000W InGaAs PIN photodiode in order to measure the pulse shape, duration, and frequency.

M2 - Animals

New Zealand white rabbits of either sex weighing 1.8 – 2.3 kg were used for the experiments. The protocol was approved by the Johns Hopkins Animal Care and Use Committee and is in accordance with the “*Guide for Care and Use of Laboratory Animals*” prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council and with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. The rabbits were

anesthetized with an intramuscular injection of xylazine (12 mg/kg) and ketamine hydrochloride (40 mg/kg). A topical anesthesia (proparacaine hydrochloride 1/2%) also was applied to each eye and a drop of homatropine bromide 5% was instilled to dilate the pupil. A dilated pupil facilitates examining the exposed corneas for minimal lesions. The anesthetized animals were placed in a conventional holder where they were positioned with the aid of a low-power He-Ne laser whose beam was aligned to be coaxial with the 1.55 μm laser beam. The eyes were positioned so that the incident beam was perpendicular to the central cornea. A removable jig attached to the optical bench was used to ensure that the anterior surface of the cornea was located exactly at the position where the beam diameter was determined. A speculum was inserted in the eye about one minute prior to exposure to hold the eye open. In order to create a reproducible tear film, the eye was irrigated with a small amount of physiological saline solution (BSS - Alcon Surgical) that was at room temperature. Irrigation was stopped about 20 sec before exposure and the excess fluid was blotted at the limbus. The corneal surface was assumed to have returned to its normal temperature at the time of exposure. One-half hour after exposure, the rabbits, still under anesthesia, were sacrificed with Beuthanasia-D (100 mg/kg) administered in an ear vein. The eyes were enucleated and examined for damage using a Nikon photo slit-lamp microscope.

M3 - Damage Determination

The criterion we use to determine minimal epithelial damage is the presence of a superficial, barely visible, gray-white spot that develops within 1/2 hour after exposure.³ Corneas were assessed for damage by examination with a Nikon photo slit-lamp. Near the damage threshold the faint diffuse spot is best observed with a slit width somewhat larger than the damage area.

In these experiments the damage threshold was well defined and there were no overlaps between exposures that produced minimal lesions and those that did not. Therefore statistical procedures such as probit analysis were not used to determine the threshold, as these would have required using more animals than necessary.^{4,7} One exposure was made per eye, initially attempting to find broadly bracketing exposures above and below threshold. The bracket was then narrowed until there was only about a 10% difference in irradiance between an exposure that produced a minimal lesion and one that did not. The injury threshold was taken to be at the center of the bracket.

M4 - Temperature Calculations

Temperature calculations are based on a time-dependent Green function solution to the heat equation for the case in which a Gaussian profile laser beam incident on a semi-infinite slab is absorbed according to the Beer-Lambert law.⁸⁻¹⁰ The calculations neglect heat transferred from the epithelial surface to the air via convection, radiation, and evaporation. This assumption was justified previously.⁶ The calculations also ignore the possibility of convection in the anterior chamber that may be produced by this penetrating radiation, particularly for exposures lasting several seconds. The thermal properties of cornea are assumed to be the same as water.^{11,12} The absorption coefficient, α , at 1.55 μm was assumed to be 12.3 cm^{-1} , which is the value for physiological saline.¹³ This value of α was used because the temperatures were calculated just under the tear layer and also to provide a direct comparison with previous studies that used the appropriate value for saline.^{4,6,11,14} The solution for the temperature increase $\Delta T(r,z,t)$, where r is the radial distance from the beam axis, z is the depth into the cornea, and t is time, has the form

of a definite integral that can be evaluated numerically. The temperature increase $\Delta T(r,z,t)$ is directly proportional to the incident irradiance. Thus we calculate $\Delta T(r,z,t)$ for an incident irradiance of 1 Watt/cm² and determine the temperature increases for different exposure conditions by multiplying by the appropriate irradiance.

Results and Discussion

The Year 1 Statement of Work was:

1. The laser will be purchased. Following receipt of the laser we will verify its power output and stability and measure its beam characteristics.
2. We will measure damage thresholds for four exposure durations less than 0.5 seconds (e.g., 0.025, 0.05, 0.1, and 0.25 seconds). The thresholds will be determined for a beam diameter of 1 mm.
3. The development of theoretical damage models will be advanced by examining the effect of including induced convection in the anterior chamber in the thermal model.
4. We will begin determining thresholds for multiple-pulse exposures.

The Year 2 Statement of Work was:

1. Statement 3 from Year 1 will continue.
2. Statement 4 from year 1 will continue.
3. We will determine the endothelial damage threshold for two single-pulse exposure durations (e.g., 1 and 10 seconds) with a 7 mm diameter beam. This is expected to require 8 rabbits.

As noted in the Year 1 annual report, we deferred characterization of the IPG Photonics model ELD-10-1550 Erbium fiber laser until Year 2. We also deferred work on items 3 and 4. This year we have installed and characterized the IPG laser, begun to determine multiple-pulse injury thresholds (Year 1 –item 4 and Year 2- item 2), recalibrated the exposure durations for the single-pulse exposures (carry over from Year 1-item 2), and completed and submitted a manuscript on the single-pulse thresholds. I also was invited to co-author a chapter on Corneal and Skin Effects of Laser Radiation with Mr. Bruce Stuck in the upcoming State of the Art Report (SOAR) on the Biomedical Aspects of Military Lasers. These accomplishments are discussed in detail below.

When the IPG Photonics laser was installed two major problems were identified. First, we found that the beam was not radially symmetric. Knife-edge scans in each of two orthogonal directions revealed that, although the profiles were approximately Gaussian, the 1/e radii in the two directions were respectively 0.191 cm and 0.248 cm. Second, we found that the laser was emitting ~60 mW when it was being maintained in the “off” position by the required 5 volt TTL control signal. This output was constant, independent of the laser power setting. Moreover, when the LabView program we wrote was used to have the laser emit a sequence of pulses, we discovered the pulse output was on top of this constant baseline. After conversing with engineers at IPG Photonics, the laser was returned to see if these problems could be corrected. Fortunately

they were able to make the necessary modifications, but resolution of these problems took three months. A new output collimator was installed and the laser driver circuit was modified. Following these modifications the output beam from the collimator was radially symmetric with an accurate Gaussian profile (1/e radii equal to 0.163 cm and 0.167 cm in two orthogonal directions) and there was no output in the "off" condition.

We have begun to determine multiple-pulse injury thresholds (Year 1 –item 4 and Year 2- item 2). To date have determined epithelial injury thresholds for sequences of 0.025 sec pulses at frequencies of 10 and 20 Hz with a nominal beam diameter of 0.1 cm. Thresholds At 10 Hz were determined for 8, 16, 32 and 128 pulses and at 20 Hz they were determined for 8, 32, 64 and 256 pulses. The determinations required 50 eyes from 25 rabbits. The measured threshold radiant exposures are compiled in Table 1.

Immediately prior to writing this report we discovered another problem with the IPG laser. As we attempted to begin multiple-pulse exposures with a larger beam diameter (which requires higher laser power), we observed that, rather than emitting sequences of equal power pulses, the first 10-20 pulses started at low power and gradually built up to the set level. We are investigating possible solutions to this new problem with the manufacturer.

Table 1: Threshold radiant exposure per pulse and calculated maximum temperature rises for sequences of pulses of Er fiber laser radiation at 1.55 μm .

Number of Pulses	Repetition Frequency (Hz)	Pulse Duration (s)	H_{th} (J/cm ² /pulse)	$r_{1/e}$ (cm)	ΔT_{max} (C) ^a
8	10	0.025	4.18	0.0492	45.2
16	10	0.025	3.08	0.0493	45.2
32	10	0.025	2.36	0.0494	43.4
128	10	0.025	1.71	0.0496	37.1
8	20	0.025	3.25	0.0492	44.6
32	20	0.025	1.63	0.0541	48.0
64	20	0.025	1.29	0.0512	45.7
256	20	0.025	0.982	0.0517	46.4

^a Maximum peak temperature rise calculated on the beam axis, 10 μm beneath the anterior tear surface.

Figures 1a and 1b show typical super-threshold lesions resulting from exposures to 32 pulses at 10 Hz. The radiant exposures per pulse for the lesion in Figures 1a and 1b were,

respectively ~60 and ~4 percent above the damage threshold. The circular lesion from the higher exposure is surrounded by an annular ring. As expected the lesion from the exposure nearer the damage threshold is smaller in diameter. The appearance of these lesions is consistent with thermal lesions produced by moderately penetrating radiation from a Tm:YAG laser at 2.02 μm .¹⁵

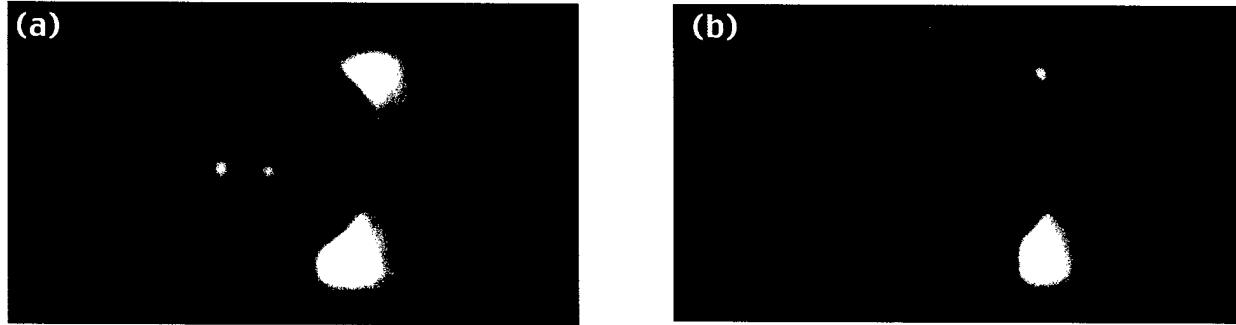


Figure 1: (a) Lesion resulting from an exposure to 32, 0.025 sec pulses at 10 Hz. The radiant exposure was 3.8 J/cm²/pulse and the 1/e beam radius was 0.0494 cm. This exposure is ~60% above the threshold exposure. The central lesion is surrounded by a diffusely scattering ring. (b) Lesion resulting from an exposure to 32, 0.025 sec pulses at 10 Hz. The radiant exposure was 2.45 J/cm²/pulse and the 1/e beam radius was 0.05 cm. This exposure is ~4% above the threshold exposure. The lesion is barely visible and is smaller in diameter than the lesion in (a).

In previous investigations it was shown that threshold radiant exposures were correlated by an empirical relationship of the form $H_{\text{th}} = CN^{-\alpha}$, where N is the number of pulses in the sequence and the exponent had values between ~0.2 and ~0.3.^{2,4,7} These results form the basis of Hypothesis 2. Figure 2 is a plot of the threshold radiant exposures from Table 1 as a function of the number of pulses. It is obvious that similar power laws also correlate these data. However, while the values of the exponent α for the two curves are nearly the same, the multiplicative constants are different, even though the duration of the individual pulses is the same for both curves. The estimated threshold for a single 0.025 sec pulse is shown by the arrow (see discussion below). Its value, 4.90 J/cm², is substantially less than either of the values for C obtained from the fits. Therefore it is apparent that the power law, although valid for $N \geq 8$, breaks down for smaller numbers of pulses where it overestimates the thresholds. This type of power law was also found to break down for some (but not all) multiple-pulse exposure thresholds for both CO₂ and Tm:YAG laser radiation; however, for these lasers the relationship underestimated the thresholds for small numbers of pulses.^{4,15}

Temperature-time histories were calculated for the threshold exposures to aid in testing the hypothesis that the injuries are based on a thermal damage mechanism and can be described either by a critical temperature damage model or by a modified critical temperature model similar to the models that describe CO₂ and Tm:YAG laser damage.^{4,6,15} As noted above the

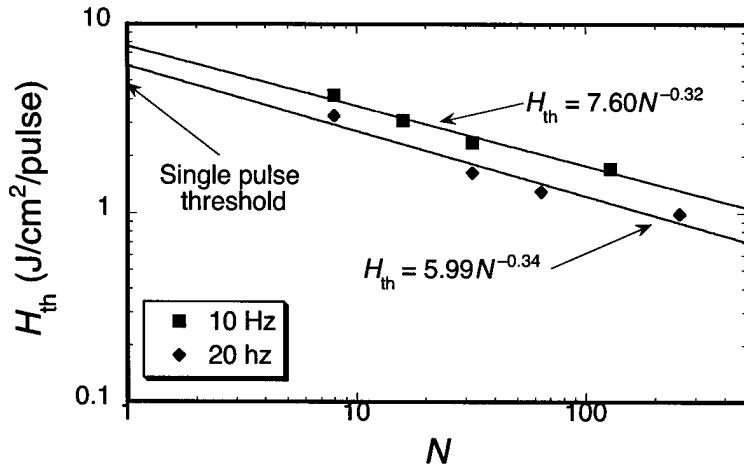


Figure 2: Dependence of the threshold radiant exposure on the number of pulses (data from Table 1). The duration of the individual pulses was 0.025 sec. The lines are least squares fit to the power law as shown on the plot. The value of R was 0.99 for the 10 Hz data and 0.98 for the 20 Hz data. The arrow shows the estimated threshold for a single 0.025 sec pulse.

calculations were done for a point on the beam axis 10 μm beneath the tear surface, which is just inside the anterior epithelial cells. Figure 3 shows calculated temperature histories for two of the threshold exposures listed in Table 1. The predicted maximum temperature increases for all of the thresholds are listed in Table 1. The mean temperature increase at the damage threshold is 44.4 ± 3.25 C (mean \pm SD). Thus the calculated temperature increases for these threshold exposures are constant to within less than 10 percent and are therefore consistent with a critical temperature damage model. This is in contrast to the thresholds for single-pulses with exposure durations < 1 sec which were discussed in the Year 1 Progress Report. The temperature rises calculated for these thresholds were not consistent with those for Er fiber laser threshold exposures having durations ≥ 1 sec or for CO₂ and Tm:YAG laser threshold exposures.

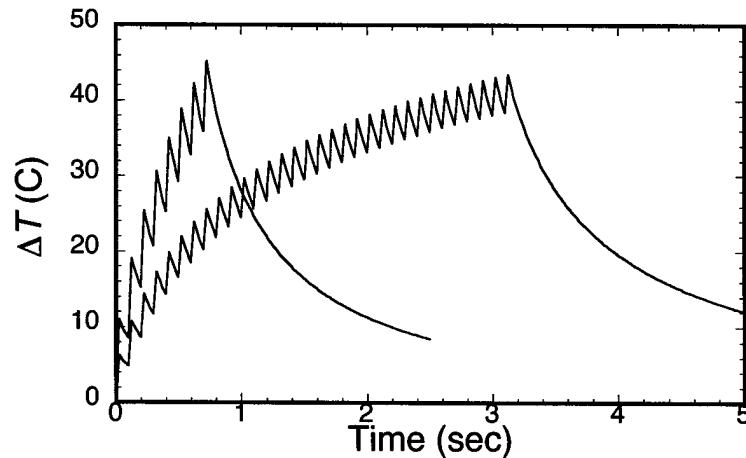


Figure 3: Temperature histories calculated for injury threshold exposures for 8, 0.025 sec pulses at 10 Hz, and 32, 0.025 sec pulses at 10 Hz. The temperature rises were calculated at a position on the beam axis 10 μm beneath the tear surface.

The fast InGaAs PIN photodiode that was built this year was used to check the calibration of the Uniblitz shutter that was used to control the pulse durations for the single-pulse exposures having durations < 1 sec that were discussed in the Year 1 Progress Report. The original calibration had been done with a slow photodiode circuit. The durations were found to be longer than those determined previously. Although this finding resulted in greater predicted temperature increases for the threshold exposures, the increases were insufficient to account for the differences with previous results for Er fiber laser thresholds for exposures > 1 sec or for CO₂ and Tm:YAG laser thresholds. Table 2 shows the revised exposure durations and their effects on the predicted temperature rises and Figure 4 shows a corrected version of Figure 3 from the Year 1 Progress Report.

Table 2 (revised from Year 1 Report): Threshold radiant exposures, threshold irradiances, and calculated maximum temperature increases at the damage threshold for single-pulse exposures.

τ (sec) Old	τ (sec) New	$d_{1/e}$ (cm)	H_{th} (J/cm ²)	I_{th} (W/cm ²)	ΔT (C) Old	ΔT (C) New
0.24	0.255	0.100	13.8	57.4	27.4	28.0
0.10	0.110	0.100	9.43	94.3	22.4	23.7
0.045	0.056	0.099	6.75	150	17.5	20.9
0.025	0.036	0.099	4.60	184	12.4	17.1

^a Calculated on the beam axis, 10 μ m beneath the anterior tear surface.

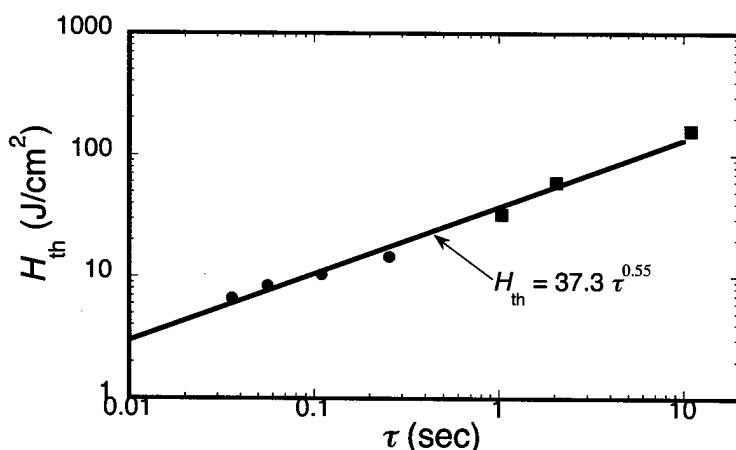


Figure 4. The circles are the values of threshold radiant exposures listed in Table 2 and the squares are threshold radiant exposures for a 1 mm diameter beam taken from reference 17. The line is a least squares fit to a power law of the form shown in the figure. The R value of the fit was 0.99.

As noted above, this year I was invited to co-author a chapter on Corneal and Skin Effects of Laser Radiation in the upcoming State of the Art Report (SOAR) on the Biomedical Aspects of Military Lasers. This *SOAR* is sponsored by the U. S. Army Medical Research and Material Command. My co-author is Mr. Bruce E. Stuck, Director of the U. S. Army Medical Research Detachment, Brooks City-Base, Texas. The chapter is a comprehensive review of effects on the cornea and skin that result from exposure to infrared laser radiation (wavelengths from 1.315 to 10.6 μm). The chapter also includes a discussion of thermal and damage models. I have completed a draft of the corneal and modeling sections and have sent it to Bruce Stuck.

--Key Research Accomplishments--

- Damage thresholds were determined for sequences of 0.025 sec pulses at frequencies of 10 and 20 Hz with a nominal beam diameter of 0.1 cm. Thresholds At 10 Hz were determined for 8, 16, 32 and 128 pulses and at 20 Hz they were determined for 8, 32, 64 and 256 pulses.
- Threshold radiant exposures for sequences of sub-threshold pulses are correlated by an empirical relationship of the form $H_{th} = CN^{-\alpha}$, where N is the number of pulses in the sequence. The exponent α is similar for the 10 and 20 Hz data, but the values of the constant C differ for the two repetition rates. Moreover the values of C are larger than the estimated threshold for a single 0.025 sec pulse.
- Thresholds for the multiple-pulse exposures are consistent with a critical temperature damage model.

--Reportable Outcomes--

Manuscripts

1. R. L. McCally and C. B. Bargeron, "Corneal Epithelial Injury Thresholds for Multiple-pulse Exposures to Tm:YAG Laser Radiation at 2.02 μ m," *Health Phys.* 85, 420-427 (2003).
2. R. L. McCally, J. Bonney-Ray, and C. B. Bargeron, "Corneal Epithelial Injury Thresholds for Exposures to 1.54 μ m Radiation," in *Proceedings of the SPIE*, Vol. 4953, *Laser and Non-coherent Light Ocular Effects: Epidemiology, Prevention, and Treatment*, 2003.
3. R. L. McCally, J. Bonney-Ray, and C. B. Bargeron, "Corneal Epithelial Injury Thresholds for Exposures to 1.54 μ m Radiation – Dependence on Beam Diameter," (submitted to *Health Physics*).

--Conclusions--

Corneal epithelial damage thresholds for exposures to sequences of pulses of 1.55 μ m infrared radiation produced by a Er fiber laser were investigated. Thresholds were determined for sequences of 8 to 128 pulses at a repetition frequency of 10 Hz and 8 to 256 pulses at 20 Hz. The duration of the individual pulses was 0.025 sec and the 1/e diameter of the laser beam was 0.1 cm. Threshold damage is correlated by an empirical power law of the form $H_{th} = CN^{-\alpha}$, where H_{th} is the threshold radiant exposure per pulse, and N is the number of pulses. This result provides confirmation of the hypothesis that a power law of this form correlates injury threshold exposures. The value of the exponent α is 0.32 for the 10 Hz exposures and 0.34 for the 20 Hz exposures. The constant C differs for the two repetition frequencies and is greater than the estimated threshold for a single 0.025 sec pulse. Thus the empirical power law breaks down for small numbers of pulses (viz., $N < 8$), where it overestimates the injury thresholds. An empirical critical temperature model also correlates the multiple-pulse injury thresholds. This result provides at least partial confirmation of the hypothesis that damage from 1.55 μ m radiation is thermal.

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